REMARKS

Claims 1-27 are pending. Amendments to the originally misnumbered claims have been acknowledged. Claims 12-27 are withdrawn as being directed to non-elected subject matter. The withdrawn claims are merely to comply with the Restriction Requirement and is not to be construed as surrender of any subject matter in the instant application. Applicants hereby reserve the right to pursue the subject matter of the canceled claims in one or more divisional patent applications.

Claim Rejections Under 35 U.S.C. § 102

Claims 1, 4-7, 9-11 are rejected under 35 U.S.C. § 102(e) as being anticipated by US 2002/0182728 (Ramiya *et al.*)

Applicants respectfully traverse.

Applicants method utilizes DMSO for 3 days then switches to a high glucose and 10% FBS media for 7 more days have **complete** transition to insulin producing cells. For example, Applicants teach on page 9, lines 19-29 through to page 10, lines 1-21:

> BM cells were collected from the femurs and tibias of rats. The marrow cells were cultured in DMEM, low (5.5mM)glucose (GIBCO cat.# 11885-084) supplemented with 10% FBS. After 60 minutes of incubation, non-adherent cells were collected and washed with serum-free DMEM medium. The cells were reinoculated in the serum-free DMEM medium at a cell density of 1 x $10^5/cm^2$ in the presence of 1% **DMSO for 3 days**. The cells were then cultured in 10% FBS-containing medium in the **high concentration glucose** (25 mM, high glucose, DMEM, GIBCO, Catalogue # 11995-065) **for 7 days.** The cells were plated in plastic 6 well plates on slide coverslips (22 x 22 mm) coated with 0.3% type I collagen, which was extracted from the rat tail tendon by the method described by Michalopoulos and Pitot, Exp. Cell Res. 94:70-78 (1975).
>
> Small spheroid clusters began to form at Day 7 under high-glucose conditions. After Day 10, the number and dimension of the spheroid cell clusters were considerably increased and formed a tightly organized mass of cells. Multiple clusters could be seen in single fields. At higher magnifications, the clusters appeared to have defined edges and structure. The 3-D cell growth morphologically resembled islet-like clusters, as

described by Bonner-Weir et al., Proc. Natl. Acad. Sci. U. S. A. 97:7999-8004, 2000; Zulewski et. al., Diabetes. 50:521-533, 2001; Ramiya et al., Nat. Med. 6:278-282, 2000; and Yang et. al., Proc. Natl. Acad. Sci. U. S. A. 99:8078-8083, 2002. The level of glucose in the media had a significant effect on the number of clusters formed by the end of Day 10. High glucose culture conditions gave a mean cluster value of 157.5±32.9 clusters per coverslip (n = 8 wells/3 separate experiments) while low glucose conditions produced 17.3±11.3 (n = 8 wells/3 separate experiments) clusters per coverslip on a 22 x 22 mm coverslip. Additionally, the cluster size under low glucose conditions was markedly smaller as compared to that of high glucose conditions.

In another culturing method, BM cells were cultured in the presence of 1% **DMSO** for 3 days, and changed to DMEM containing 4.5 g/L glucose with 10% FBS for **7 days**. To enable the detection of insulin secretion without interference from the fetal serum, the medium was then changed to serum-free medium. The serum-free medium was supplemented with 0.5% bovine serum albumin (BSA) and 5.5 mM glucose. The BM cells were incubated in the serum-free medium for 5 hours at 37°C and washed twice with serum-free medium. The media was then changed to media containing high glucose (e.g., 25 mM) for 2 hours and the cells were incubated at 37°C. The culture-conditioned media were collected and frozen at -70°C. (Emphasis added).

Applicants therefore describe the production of insulin producing cells in the presence of DMSO, low and high glucose within 7 days. Applicants further describe the phenotype of the cells after 7 days of culture, on page 12, lines 1-25:

Immunofluorescent staining of pancreas tissue subsequent to transplantation and a copper-deficient diet using DPPIV-FITC and Insulin-Texas Red as markers was performed. Untreated DPPIV+ rat liver and pancreas were examined as controls. Insulin cells from an untreated DPPIV-deficient pancreas were not DPPIV+. There was no staining for DPPIV in either the untreated DPPIV-deficient pancreas or the untreated DPPIV-deficient liver. However, the beta cells did stain positive for insulin. Male rats were lethally irradiated and rescued with a BM transplant from a male animal. Engrafted male rats were then placed on the copper deficient diet protocol for a six-week period and placed back on a normal diet. Immunohistochemical localization of insulin and DPPIV in pancreas, after transplantation and copper deficient protocol (6-week time point) was analyzed. Two markers were used: DPPIV-FITC, Insulin-Texas Red. (A) Immunofluorescent staining of pancreas with insulin antibody, (B) Immunofluorescent staining of pancreas with DPPIV antibody. (C)

> Merge imaging of DPPIV expression in cells which co-expressed insulin. Figure 1 shows expression of DPPIV cells in pancreas of BM transplanted animals. DPPIV expressing cell were counted at 2, 4, 6, 8, and 10 weeks in pancreatic tissues. In each of the BMTx DPPIV- deficient males, 60 sections were cut from frozen pancreas, stained and counted for the total number of DPPIV positive cells. White bar represents total DPPIV expressing cells and black bar represents DPPIV expressing cells in Langerhans Islet. Numbers are shown as percentages of Langerhans islet cells/ total cells that were DPPIV positive.
>
> Immunohistochemical techniques were also used to evaluate expression of insulin in BM cells cultured in vitro. Immunohistochemical localization of insulin in BM cells differentiated into pancreatic lineages in vitro was analyzed. Immunofluorescent staining with insulin and nuclear staining of a) control BM cells and b) BM cells cultured in 1% DMSO after 10 days was performed. Unlike the control BM cells, the **cells cultured in 1% DMSO formed spheroid clusters and stained positive for insulin.** (Emphasis added).

Applicants show that culture of the bone marrow cells in 1% DMSO differentiate into insulin producing cells.

In contrast, Ramiya *et al.*, **do not** teach or use culture of the bone marrow cells in **1% DMSO** for 7 days nor the sequential use of "low" and high glucose media for the trans-differentiation of non-pancreatic cells and for stimulation of endocrine hormone production. Ramiya *et al.*, discuss a **cocktail** that includes a many specific cells factors, see for example Tables 1A and 1B. Ramiya *et al.*, also discuss the need for the cells to be **cultured for 14 days using basal media of Table 1A** and then a further **culture in the cocktail of Table 1B for 21 days.** Thus, Ramiya *et al.*, neither teach nor anticipate Applicants invention.

In view thereof, Applicants respectfully request reconsideration and withdrawal of the instant rejection.

Claim Rejections Under 35 U.S.C. § 103

Claims 1-11 are rejected under 35 U.S.C. § 103(a) as being unpatentable over US2002/0182728 (Ramiya *et al.*) taken with Yang *et al.* (IDS reference; *PNAS*, June 2002, Vol.

99, No 12, pages 8078-8083), Petersen *et al.*, (IDS reference, *Science*, May 1999, Vol. 284, pages 1168-1170), US 2003/0104997 (Black *et al*, and US 6,458,589 (Rambhatla *et al.*)

Applicants respectfully traverse.

Applicants have discussed Ramiya et al above. In summary, Ramiya et al., **do not** teach or use culture of the bone marrow cells in **1% DMSO** for 7 days nor the sequential use of "low" and high glucose media for the trans-differentiation of non-pancreatic cells and for stimulation of endocrine hormone production.

Yang *et al.*, further **do not** teach the **sequential** use of low and high glucose medium in the presence of **DMSO**. Yang *et al.*, discuss adding 23 mM glucose to stem cells maintained in a **cocktail** of factors, e.g., LIF, stem cell factor, Flt-3 ligand before adding glucose 6 months later. Furthermore, Yang *et al.*, discuss that small spheroid cultures began to form after **at least two months** culture in high glucose medium. (See, col. 8079, par. 4, first few lines). Yang therefore, fails to cure the deficiencies of Ramiya *et al.* The combination of Ramiya, Yang and Petersen further do not teach Applicants invention. Petersen does not teach or disclose culturing of bone marrow cells in DMSO and sequential culture in low and high glucose media to differentiate the bone marrow cells into pancreatic hormone producing cells. In addition, neither of the references or combination thereof, provides any motivation or indicia of reasonable success for the use of DMSO and sequential use of low and high glucose media to differentiate the bone marrow cells into pancreatic hormone producing cells.

Black et al., discuss DMSO however, do not provide guidance as to amounts, time points nor the combination of DMSO, 10% Fetal calf serum, sequential low and high glucose conditions for 7 days. The combination of Ramiya, Yang, Petersen and Black do not teach Applicants invention.

In view thereof, Applicants respectfully request reconsideration and withdrawal of the instant invention.

CONCLUSION

Applicants respectfully request entry of the foregoing remarks and reconsideration and withdrawal of all rejections. It is respectfully submitted that this application with claims 1-11 define patentable subject matter and is in condition for allowance. Accordingly, Applicant respectfully requests allowance of these claims.

This response is being filed within the shortened statutory period and thus believe that no fees are due. Although, Applicants believe that no extensions of time are required with submission of this paper, Applicants request that this submission also be considered as a petition for any extension of time if necessary. The Commissioner for Patents and Trademarks is hereby authorized to charge the amount due for any retroactive extensions of time and any deficiency in any fees due with the filing of this paper or credit any overpayment in any fees paid on the filing or during prosecution of this application to Deposit Account No. 50-0951.

If there are any remaining issues or the Examiner believes that a telephone conversation with the Applicants' attorney would be helpful in expediting prosecution of this application, the Examiner is invited to call the undersigned at telephone number shown below.

Respectfully submitted,

AKERMAN SENTERFITT

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